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THE LIFE HISTORY OF DESMOGNATHUS FUSCA.

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Although *Desmognathus fusca* ranks as one of the commonest of the American salamanders, at least in the eastern part of the United States, our published accounts of its life history are singularly meager and fragmentary. It is hoped, therefore, that the following account, culled from observations extending through several years during which this species has served as material for class work in anatomy, histology, embryology, and physiology in our laboratory courses at Smith College, as well as for several lines of individual research, may prove of some interest and even practical value to others who are engaged in the study of amphibian life. Incidentally, too, there is always the possibility that facts presented for their intrinsic interest may prove to have some broader biological significance, particularly in a field like that presented by the amphibians, in which there are involved, both phylogenetically and ontogenetically, so many environmental transitions which call for adaptive response.

SYSTEMATIC POSITION AND SPECIFIC CHARACTERS.

Desmognathus fusca is a representative of the family Desmognathidæ, which, together with the family Plethodontidæ comprises all of the American Urodeles now known to be lungless.¹ In common with the other members of these two families, *Desmognathus* possesses the naso-labial groove (Whipple, '06a) and lacks the ypsiloid cartilage (Whipple, '06b).

The following description of the external appearance of *Desmognathus fusca* is given by Morse ('04, p. 115): "*Desmognathus fusca fusca* Raf. Above dark, with a median dorsal band of lighter generally brown in color and specked with black. Below gray with black specks forming a marbling. . . . Very variable in color. Costal grooves 14. Length 4½ inches."

¹ *Ambystoma opacum*, reported lungless by Lönnberg, has since been shown to possess fully developed, functional lungs (Whipple, '06b).

As pointed out by H. H. Wilder ('99), *Desmognathus fusca* may be conveniently distinguished from *Speleopetes bilineatus*, the other lungless species with which it is usually associated in habitat, by its more robust body, its general darker color, and less distinct markings. In *Desmognathus* the markings become more and more obscure with the increasing size and darkening color of the older adults. In many even of the smaller adult *Desmognathus*

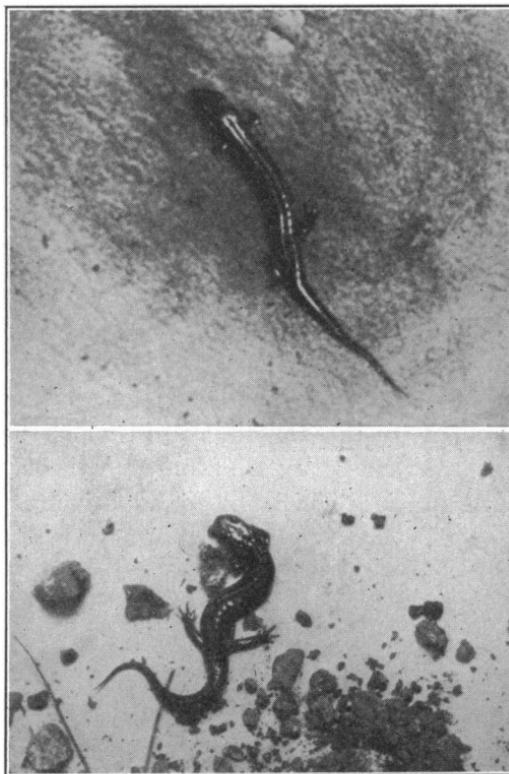


FIG. 1. Photographs of living adult *Desmognathus fusca*, in two characteristic attitudes, about one half natural size.

individuals the whole color becomes very dark and the ventral surface thickly mottled with pigment spots, while *Speleopetes bilineatus* remains practically unpigmented ventrally. The larvae of the two species are, however, even more frequently confounded. Their differences have been clearly shown by

Wilder ('99). *Desmognathus fusca* larvæ undergo metamorphosis while still very small, from 28 to 30 mm. in length (although the duration of larval life is much longer than Wilder's estimate), while *Spelerpes bilineatus* may attain before metamorphosis a length of over 50 mm., greater indeed than the length of certain adult specimens. The *Desmognathus* larvæ may be distinguished from the younger *Spelerpes* larvæ by the proportionate size of the hind legs, which in *Desmognathus* are from the time of hatching much larger and stouter than the fore legs and are held when at rest at a considerable angle from the body, while the hind legs of the small *Spelerpes* larvæ are slender and scarcely exceed the fore legs in length. The digits of both fore and hind feet of *Spelerpes* larvæ of all sizes are attenuated, and the feet never have the robust appearance of those of *Desmognathus* larvæ.

Morse ('04) says of *Desmognathus fusca*: "The larvæ attain a length of three inches before becoming mature, and inhabit springs and small bodies of water. They are brown with black dots above, lighter below, retaining also the black dotting. The gills are short and inconspicuous. Sometimes the black is dotted here and there with livid specks of red, but this disappears in the adult condition." This is undoubtedly a case of incorrect identification as the description does not correspond to *Desmognathus fusca* larvæ in the matter of size, color, or habitat. I am able to refute the identification with considerable positiveness for the reason that my conclusions concerning every stage of the life history of this species have been corroborated by careful study for several successive years of material obtained from a much circumscribed little stream in which *Desmognathus fusca* is the only salamander that occurs.

HABITAT.

As compared with other Urodeles, *Desmognathus fusca* may well rank as one of the more terrestrial. Its lungless condition is in itself an indication of its terrestrial habitat; for, as pointed out in a previous paper (Whipple, '06b), amphibian lungs serve an important hydrostatic function, so that with the exception of certain lungless forms such as *Spelerpes ruber*, which have

secondarily adapted themselves perfectly to aquatic life at the bottom of the water, the lungless condition unfits an amphibian for a prolonged stay in the water. If further evidence on this point is desired one has only to drop living specimens of *Diemyctylus viridescens* and *Desmognathus fusca* into a deep aquarium and compare their behavior!

The lungless salamanders show, however, varying grades of environmental adaptation, from *Spelerpes ruber* above cited as a perfectly aquatic form, to *Plethodon cinereus*, which is completely



FIG. 2. Photograph of a typical habitat of *Desmognathus fusca*, as it appears in late autumn, when the water in the little brook is for the most part covered with fallen leaves, which serve as a protection to both the larvæ and the adults.

terrestrial even during its larval life, while the extreme is reached in the case of *Autodax lugubris*, reported by Ritter and Miller ('99) to be practically arboreal throughout its entire life.

Desmognathus fusca is terrestrial to the extent that it lives in and upon moist earth, where it mates and lays its eggs, and

where the first few days of the larval life are spent. The young larvæ, however, soon reach shallow running water where they remain for nearly a year, after which they come out of the water to live the terrestrial adult life of the species. The presence of aquatic as well as terrestrial insect forms in the stomachs of adult *Desmognathus* shows, however, that even the adults are by no means absolutely terrestrial, but, so far as my own observations and those reported by others go, the adults confine their aquatic excursions to very shallow water, and they are seldom found in the water at all unless driven there.

Apparently, the ideal environmental conditions for *Desmognathus fusca*, as deduced from a study of those localities in which they occur in the greatest abundance, are those afforded by the banks of well shaded streams of shallow, perennially running water. In such places they may usually be found under every stick and stone and fallen log at or below the water level, while the soft earth is riddled with burrows, often, it is true, the work of earthworms or of other animals, but utilized as convenient lurking places for *Desmognathus*; for the adult *Desmognathus* is, in the day time, at least, thigmotropic, and will even take refuge in a bottle left lying on the surface of the soil exposed to the light, usually orienting the body with the head toward the mouth of the bottle. *Desmognathus* has the power to make its own burrows, as it will demonstrate, sooner or later, when isolated in a terrarium which affords no other suitable means of protection. In one case in particular which came under my observation, two individuals were accidentally set aside in a terrarium which was supposed to contain no specimens. Over a year afterwards it was found that these animals were still living in this terrarium under almost perfectly dry conditions. In the soil were many well formed burrows, within which the specimens, which were kept for some time after they were discovered, remained, often completely concealed, but at times with the head protruding from the entrance to the burrow.

Under natural conditions the crevices and burrows inhabited by *Desmognathus* are, of course, very moist, and I have usually been able to demonstrate, by following them carefully through their windings and branchings, a connection with the water of then eighboring brook.

The part which the shaded condition plays in the habitat of *Desmognathus* is undoubtedly a somewhat complicated one, possibly determining conditions for the necessary food supply, as well as helping to maintain a moist condition of the soil, supplying an abundant and annually replenished loose earth from fallen leaves and twigs; those leaves, also, which fall into the water furnish lurking places in which the larvæ take refuge and seek their food. The element of close proximity to running water in the habitat of *Desmognathus* is certainly not necessary to the immediate physiological demands of the adult, but is incident rather to the aquatic nature of the larval life, which makes necessary not only easy access to water after hatching, but also requires that the supply of water shall be perennial, since each year the newly hatched larvæ reach the water at about the time when the brood of the previous summer leave the water as very small adults. Upon the other hand, these small adults upon leaving the water find themselves in the midst of the proper external conditions for their whole adult life, including mating and egg laying, and therefore, so far as is known, there is no tendency whatever to annual migrations such as has been shown to occur in *Ambystoma punctatum*, for example (Wright, '08).

MATING HABITS.

In his valuable discussion of the breeding habits of amphibians, Smith ('07) has pointed out an interesting gradation in methods of fertilization from the lavish method of typical aquatic fertilization possessed by *Cryptobranchus*, in which the unfertilized eggs are expelled into the water in large numbers, to take their chances at being fertilized by the abundant spermatic fluid expelled by the male in their vicinity, to the opposite extreme of typical internal fertilization without spermatophores, which the *Apoda* have been shown to have acquired as an adaptation to their completely terrestrial existence. Between these two extremes lie such forms of fertilization as that of the *Ambystoma punctatum*, in which the spermatozoa are not poured freely into the water, but are enclosed within spermatophores which the male, stimulated by the presence of the ripe or nearly ripe female, deposits in considerable numbers, leaving with the

female, however, the whole responsibility for the entrance of one of these into her cloaca (Wright and Allen, '09); and the still more certain method of the various *Tritons*, in which the male uses special devices, such as clasping, to ensure the entrance into the cloaca of one of the small number of spermatophores, thus making certain the fertilization of a far larger proportion of the reduced number of eggs. Smith ('07) points out that in the increasing economy in the amount of seminal fluid shown by such mating habits as those of the *Ambystoma* and *Triton* we see, as an incidental result, a preparation for possible terrestrial life.

In *Desmognathus fusca* we see a form which has availed itself of this, as well as of other possibilities of adaptation to terrestrial existence; for, although the larvæ retain the typical aquatic nature of amphibians, the habit of a periodical return to the water for the mating and egg laying which an aquatic larval life usually involves has been abandoned, and both of these functions are performed by the adults without the inconvenience of leaving their terrestrial abode. My information concerning the actual act of mating is drawn from a single observation. Since, however, both Kingsbury ('02) and Hilton ('09) state that nothing definite seems to have been published upon this point, I feel warranted in giving a full report of this single observation.

On the evening of May 13, 1908, I isolated in a small terrarium a large male, and a female through the abdominal wall of which large eggs could be seen. It was discovered the next morning, however, that another smaller male was also present in the terrarium, probably having been carried over unobserved in transferring some wet leaves. On the following morning, May 14, the female and this smaller male were found lying upon the earth under some wet leaves, the ventral surfaces of the bodies in contact. They reacted so quickly, however, to the disturbance of the leaves that beyond this very hasty observation as to their general position I can state nothing definite as to methods of clasping or exact regions of contact. Protruding from the cloaca of the female was a yellowish, semi-fluid mass which was found upon examination to be a spermatophore of very soft consistency.

When placed in a drop of water upon a slide, the spermatophore

was found to contain large numbers of tightly coiled spermatozoa (Fig. 3, *x*), which, however, in a few minutes began to uncoil, so that in the course of half an hour the majority were uncoiled or uncoiling. Some few, however, were still coiled seven hours later. From time to time some of the spermatozoa were seen to be coiling up again, a condition which was probably induced, as was shown by a subsequent study of spermatozoa taken from

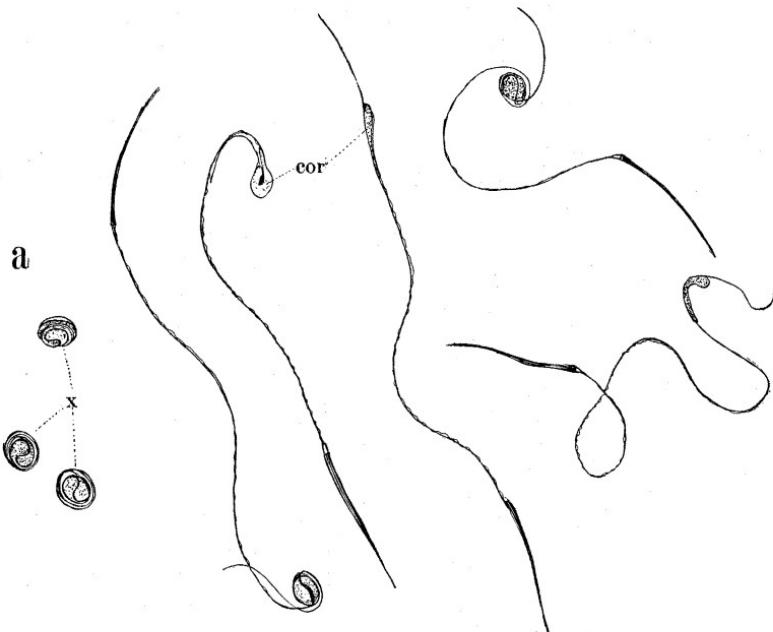


FIG. 3. Spermatozoa of *Desmognathus fusca*, showing method of coiling; *cor*, the mass or corpuscle about which the spermatozoa coil; *x*, spermatozoa coiled as found in the spermatophore. Based upon drawings with Abbé camera. $\times 290$.

the ducts of living males, by partial drying of the preparation. All of these movements were somewhat jerky and mechanical, and none of the free swimming movements such as the living spermatozoa exhibit within the ducts of the male were to be seen. It must be noted, however, that the conditions were not perfectly normal, since the spermatophore, obviously not intended for an aquatic medium, was placed in water for examination. In the case of another spermatophore, found in the cloaca of a similarly isolated female, and examined microscopically without the

application of water, the spermatozoa were coiled and exhibited none of the uncoiling movements. In this case the spermatoaphore was of a much firmer consistency, which, as no male was found with the female, may have been due to the longer lapse of time after its transfer to the cloaca of the female.

Kingsbury ('02) concluded from the presence of spermatozoa in the cloaca of the females captured at all seasons of the year, that the fertilization is internal, and further inferred from the condition of the ducts and cloaca of the male at various seasons, that the fertilization takes place not only in the spring but possibly also in the fall. The whole subject is one which demands a careful series of observations and experiments, which are, however, rendered somewhat difficult, as pointed out by Kingsbury, by the retired nocturnal habits of the animal. The above report is at least sufficient to show that the mating in this species takes place under terrestrial conditions and that the fertilization is an internal one, facts which are quite in accord with the general adaptation to terrestrial life shown by *Desmognathus*.

In my examination of spermatozoa I have invariably noted a certain appearance of which I have as yet found no mention in the literature upon the subject. Spermatozoa taken in a living and active condition from the ductus deferentia of a decapitated male, as well as those removed from the cloaca of the female, may be seen to constantly coil and uncoil, assuming in the process such appearances as are shown in Fig. 3. In uncoiling, however, there is gradually revealed an elongated or oval mass (*cor*), apparently of semifluid consistency and of a translucent material, which seems to form the core about which the coiling takes place and which is enclosed in the last final loop of the tail as it uncoils. As spermatozoa within the tubules of the testis do not possess this structure, it is apparently acquired in the passage from the testis into the duct, and is very likely a secretion of mucous nature derived from certain specialized cells. Its nature and source, however, as well as its function require further investigation. If it is peculiar to *Desmognathus*, it may well be an adaptation to the prolonged period which must often elapse between the introduction of the spermatozoa into the spermathecae of the female and the actual fertilization of the eggs,

and is one of the many devices which it is necessary for a terrestrial form to develop to resist the drying action of the air.

EGG LAYING HABITS.

Concerning the egg laying habits of *Desmognathus fusca* we have several published reports. According to Hilton ('09), the eggs are found most abundantly from the last of June to the middle or last of July. Kingsbury ('02) reports eggs of *Desmognathus* found at Ithaca during July and August, and refers to the statement of Sherwood ('95) that they have been found from July to October. Reed and Wright ('09) give July as the time of maximum egg laying. In western Massachusetts, in the immediate vicinity of Northampton I have found eggs as early as the eleventh of June and as late as the twenty-fourth of September, although, since in the latter instance the eggs were just at the point of hatching, they were probably deposited during the latter part of August. As reported by H. H. Wilder ('04), eggs have been deposited by individuals in captivity in the Smith College laboratory as early as June first. It is thus safe to state that the egg-laying period extends at least from the first of June to the last of August and possibly through September. As suggested by Hilton, temperature and humidity may have much to do with determining the exact time, and it should be further noted in this connection that the latest brood of my own observations, that of September 24, 1907, occurred at the end of a singularly cold summer, and may have been belated from that cause.

The eggs are laid in a small batch consisting usually of two masses and numbering about 20 in all (15-20 according to Hilton), a number which corresponds in general also to the count of the ripe eggs found in the ovaries of a large number of females which I have examined for purposes of comparison. The total number is practically constant, being 10 or 11 in each ovary. A few show a smaller number or a greater inequality, such as the case of 9 in the left and 5 in the right ovary, or of 13 in the left and 4 in the right ovary.

The small number of eggs deposited at a time as compared, for example, with several hundred reported for *Cryptobranchus alleganiensis* (Smith, '06, '07 and '12), 130-225 for *Ambystoma*

punctatum (Wright and Allen, '09), 108 for *Diemyctylus viridescens* (Jordan, '91), and 150 for *Amphiuma means* (Hay, '88 and '90), shows a striking correlation with the far greater certainty of fertilization which is insured by the direct deposit of the spermatophore in the cloaca of the female; the small number of eggs is also undoubtedly correlated with the superior chances for successful development afforded by the large size of the egg (about 3.5 mm. in diameter), and the maternal care during development.

The eggs are laid in a little moist hollow or cavity, often of accidental occurrence, in just such places, in fact, as those in which the adult *Desmognathus* is usually found. I have observed that the female in captivity will sometimes excavate in the soft earth underneath a stone or clump of moss a cavity sufficiently large to accommodate her body in a coiled position, and in this cavity will deposit her eggs. In nature I have found eggs under a mere covering of moss or a little decaying stick. Hilton ('09) reports that they are found also at a depth of as much as four feet below the surface. They seem never to be deposited more than two or three feet from the edge of the water, or more than a few inches above its level in the neighboring brook, into which the newly hatched larvæ are destined to find their way.

So far as my own observations and those reported by others go, the eggs are laid during the night or in the early morning, as would indeed be expected from the nocturnal habits of the species. No cases have been reported of the prolongation of the process of egg laying beyond the single night during which, apparently, all the eggs produced by a given female for one season are deposited.

The eggs are always found guarded by a female, undoubtedly the mother. She usually so places herself among them as to bring practically all of the eggs in contact with her body, which often extends through the mass of eggs and is frequently bent sharply upon itself as if the better to surround and protect them. When under observation, as in a terrarium, the mother frequently leaves the eggs when disturbed, always retreating through the same exit from the nest. After having been separated from the eggs, however, as may occur in making a transfer from out of

doors to the laboratory, the mother goes back to them again, even though the nest and all of its surroundings may have been reconstructed. I have never had the opportunity to further test the sense of ownership of eggs in a mother by exchanging the eggs of two individuals, but the experiment would certainly be an interesting one.

The function of the mother in incubating the eggs is probably mainly that of insuring the proper degree of moisture, since eggs when removed from the mother and kept moist undergo normal development. It is possible, also, that she may guard them from inundation; for the first eight or ten days eggs will develop normally, even though quite immersed in water, but during the later development a short immersion in water kills them, apparently through a rapid change in osmotic pressure between the cells of the embryo and the fluid which by this time surrounds the embryo within the protective envelopes.

I have no evidence that the habit of eating the eggs, such as Smith ('07) found in the case of *Cryptobranchus*, is ever indulged in by *Desmognathus*, although, as will be shown below, this species is by no means to be exempted from the charge of cannibalism.

THE EGGS.

The egg membranes, already very carefully described by previous writers, are three in number, the outer one being continued into a stalk which connects the eggs with the others, and is thus a common membrane for the whole mass. Smith, ('12) in comparing the egg strings of *Cryptobranchus allegheniensis* with the arrangement of the eggs of other Urodeles, suggests the probability that the most closely related egg arrangement is such as is shown by the stalked egg enclosures of *Desmognathus* rather than in the jelly-enclosed masses of certain other forms. The accompanying drawings (Fig. 4) of the two egg masses of a batch of eggs studied by me suggest the probability of the derivation of their arrangement from a more primitive rosary arrangement, such as *Cryptobranchus* possesses. A few eggs are still seen in the main axis of the bunch, but the majority have pushed out to one side, as if through an excess of lateral pressure brought to bear upon the outer envelope from within. Each

egg has thus acquired a short stalk which connects it with the other eggs of the bunch. Whether this is a change which takes place in each individual case during the descent of the eggs through the oviduct and the subsequent reaction upon exposure to air, or whether this is a form of egg string which has been derived phylogenetically from some ancestor in which the egg string was in the rosary form, I am unable to say. The stalk of each egg becomes eventually very much twisted, probably a secondary condition brought about by the movements of the

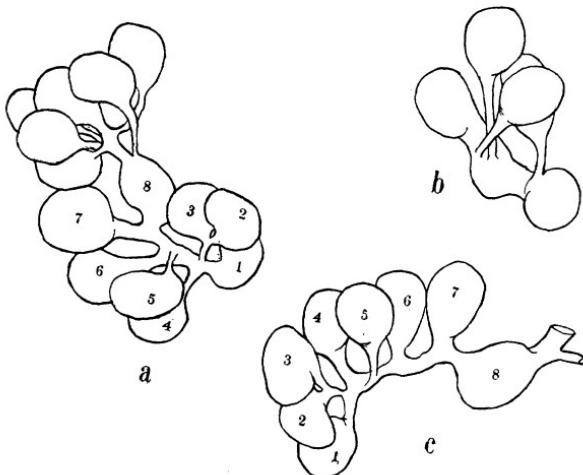


FIG. 4. Sketches of egg capsules of *Desmognathus fusca* after the larvæ have hatched; (a) and (b), the two masses of the same batch of eggs as is shown in Fig. 6; (c) a view of the egg capsules numbered 1-8 of (a), so arranged as to show their probable sequence with relation to the main stalk of the mass, and suggesting the derivation of the form of egg mass from a more primitive rosary form. Approximately twice life size.

mother among the eggs while brooding them; this twisting tends to increase the length and diminish the diameter of the stalks, thus crowding the points of their mutual attachment nearer together and emphasizing the relationship expressed by H. H. Wilder's comparison of the whole mass of eggs to a bunch of toy balloons.

The eggs themselves are creamy white, unpigmented spheres, each measuring from 3.5 to 4 mm. in diameter. This large size provides, of course, for a more advanced stage of development at the time of hatching and thus insures the preservation of a

very large percentage of the brood. The large size of the eggs, the certainty of fertilization which the internal method insures, the well-sheltered position in which the eggs are placed, and the maternal care during development are thus all conditions which compensate for the very small number of eggs.

Within the protective envelopes, the eggs orient themselves, after the manner of amphibian eggs, with the animal pole above they continue this orientation until the embryos reach a sufficiently active stage to introduce other factors into the determination of their position in the egg.

EMBRYONAL DEVELOPMENT.

Without attempting to enter into the details of the embryology of *Desmognathus*, certain general features of the course of development of the egg are in place in an account of the life history of the species.

The early development of *Desmognathus* eggs has been worked out by both H. H. Wilder ('04) and Hilton ('04 and '09). The segmentation is found to be holoblastic, although, as demonstrated by Hilton, the large size of the yolk mass prevents the complete division internally until after the early blastula stage is reached, thus placing the egg on the border line between the holoblastic and meroblastic types, while throughout its entire development the appearance of the embryo as it lies at first upon, and later coiled around, the large mass of yolk cells, so strongly suggests the condition found in meroblastic eggs that one instinctively speaks of "embryo and yolk" (Fig. 5). This resemblance is emphasized by the fact that the conspicuous capillary network which early develops on the surface of the mass of yolk strongly suggests the yolk circulation of meroblastic forms. Wilder ('04) noted the correspondence externally of certain of these blood vessels with the lateral cutaneous and median abdominal veins of the adult, and on this ground did not consider them the homologues of the vitelline veins of meroblastic forms. Piersol ('09), on the other hand, describes a similar meshwork of blood vessels in the splanchnic mesoblast of the embryo of *Plethodon cinereus erythronotus* as a vitelline circulation, and as sections of *Desmognathus* embryos show the blood vessels in question to be

similarly situated in the splanchnopleure (Fig. 11, *vbv*), there would seem to be no reason why they should not be considered the homologues of a true vitelline circulation.

The segmentation process results in the distribution of the yolk granules to the cells of the blastula, and there early appears, according to Hilton ('09), a differentiation of the cells into those containing large and those containing small yolk granules, the latter arranging themselves in a superficial layer surrounding the former, which form the whole central portion of the mass. Wilder ('04) found that the late blastula stage is reached in

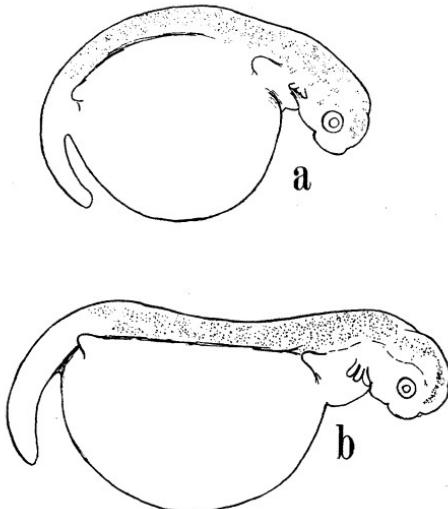


FIG. 5. Two embryos of *Desmognathus fusca* removed from two of the same batch of eggs after 16 days of development, showing a difference in the rate of development. Drawn with Abbé camera. $\times 9$.

about three days, while Hilton reports the formation of the yolk plug stage in about forty hours from the time the eggs are laid. This discrepancy may, however, be due to an individual difference in the rate of development, since Wilder found existing simultaneously in the same batch of eggs, four-, eight-, and sixteen-cell stages.

By the eleventh day both the anterior and the posterior ends of the embryo are well lifted above the surface of the yolk, and the dorsal region of the body appears as a narrow welt or ridge stretching between them. By the thirteenth or fourteenth day

the embryo begins to make spontaneous movements, forcibly bending the free anterior and posterior ends of the body to right and left. By this time the limb buds have appeared, and the dorsal surface of the embryo assumes a decidedly grayish hue, owing to the beginning of pigmentation, which rapidly increases as development goes on, and takes the form of a decided pattern by the twenty-fourth day.

External gill bushes may be readily distinguished as early as the sixteenth day. On the twenty-fifth day, however, neither the mouth nor the gill slits have opened to the exterior. On the thirtieth day the mouth has opened by two lateral slits but is still closed in the middle region. At this time, also, the two most anterior gill slits upon each side are open, the more anterior being just posterior to the gular fold.

A considerable accumulation of liquid early appears about the embryo, and continues to increase in amount, distending the egg envelopes, which are thus placed under a constantly increasing tension. From this cause it becomes increasingly easier to remove the embryo from the egg membranes, until by the eighteenth day a puncture of the membranes is followed by a rapid expulsion of the embryo through the force of the escaping fluid. This is undoubtedly a preparation for the easy rupture of the membranes and escape of the embryo when the time for hatching arrives, the fluid serving meanwhile to equalize the external pressure upon the delicate embryonal tissues and prevent injury to them.

Thirty days is the longest time for which the continuous development of any batch of eggs has been reported (H. H. Wilder, '99), so that as to the exact duration of the period of development within the egg I have very limited data. It is seldom that one knows the exact date when a given batch of eggs is deposited, or, knowing this date, can carry on the observations successfully under artificial conditions to the time of hatching. Hilton states that the rapidity of development depends upon humidity and temperature, but gives no data whatever concerning the duration of the whole period. Wilder describes the thirty-day embryo above referred to as a fully developed larva in all respects except the still large yolk mass. "The pigmentation was com-

plete, the external gills fully developed, and the feet had the full number of distinct toes (4 anteriorly and 5 posteriorly)." This embryo was 13 mm. in length, 2 mm. shorter than the shortest newly hatched larva which I have measured, and Wilder estimated that its supply of yolk might indicate a continuation of development within the egg for several days longer. Thus the length of the entire period might be estimated at approximately 5 weeks.

The length of the period of development within the egg varies, however, even in the same batch of eggs, since the hatching process sometimes continues for four or five days. This difference in the time of hatching may possibly be due to the element of chance in the occurrence of sufficient friction to rupture the membranes; since, however, the newly hatched larvae have, so far as my observations go, reached the same stage of development, though differing slightly in size, it is more probable that this difference in the date of hatching of members of the same brood is due to an actual difference in the rate of development. The difference which Wilder found in the segmentation stages of eggs of the same brood, which might be logically accounted for by a slight difference in the exact moment of fertilization, would in itself be insufficient to account for so great a difference as four or five days in the time of hatching, or for the decided difference which I have observed in the development of embryos taken simultaneously from the same batch of eggs during the latter part of the embryonal period (Fig. 5, *a* and *b*). Piersol ('09) noted a similar difference in the rate of development and consequent time of hatching in the eggs of *Plethodon cinereus erythronotus*, and attributed it to a difference in the accessibility to a supply of oxygen, the eggs on the outside of the mass having the better chance. In the case of *Desmognathus*, however, the eggs are so arranged and so frequently shifted in relative position by the movements of the mother, that it is hardly possible that they differ greatly in their access to either moisture or oxygen supply. Hilton noticed that the smaller eggs of the mass usually develop more rapidly, a condition which might readily result during the early stages from the smaller mass of inert yolk material upon which to expend the energy of cell division, and possibly

in the later stages from the larger ratio of surface for absorption, to mass.

HATCHING, AND THE TERRESTRIAL LARVAL PERIOD.

Since the development and hatching of the egg take place under terrestrial conditions, it follows that there must be a terrestrial larval period between the time of hatching and the time when the larvæ reach the water and enter upon their aquatic larval life. Concerning this terrestrial larval life of *Desmognathus* and the important changes which the transition from terrestrial to aquatic conditions naturally involves, there is, so far as I know, no published account. For this reason I shall give somewhat in detail an account of a case of the hatching and subsequent development of a brood of *Desmognathus* larvæ which came under my observation.

The batch of eggs in question were found on September 24. They were found in the usual sort of location, under a decaying stick about three feet from the edge of the water of a certain shallow brook in which *Desmognathus* abounds. A large female was, as is usually the case, coiled about the eggs, while nearby under the same stick was a smaller adult, which proved to be a male. As it is not uncommon to find two or more individuals under the same stick or stone, this fact has probably no significance. The eggs contained large and very active embryos, each still distended with a conspicuous mass of whitish yolk.

As I had not the implements at hand for removing a sufficient mass of the loose earth underneath the nest to avoid disturbing the eggs, I carefully replaced the stick and left the eggs and the adult undisturbed, intending to return immediately and remove them. This a heavy shower prevented, and the nest was not revisited until the next morning. The female was found under the stick two or three inches from the eggs, which had already begun to hatch. She was in a rather poorly defined burrow and was headed away from the eggs. The whole family (mother with the hatched larvæ and unhatched eggs) was taken up upon a mass of soil included in a radius of about five inches and several inches deep, and was carefully transferred to the laboratory. The mother made no attempt to escape and during the journey

returned to the eggs and placed herself among them (Fig. 6). Three larvæ and fifteen unhatched eggs were counted at this time; it is probable that two larvæ had already escaped from the nest, however, as a subsequent examination of the egg envelopes after all had hatched showed the entire number of eggs to have been twenty. The larvæ were very active and crawled about over the moist body of the mother; when disturbed they made quick jerky jumps among the loose debris of pine needles and decaying leaves upon which the eggs were lying. Two of the larvæ were killed at this time and were found to measure 15 and 15.5 mm. respectively (stage A of the subsequent description).

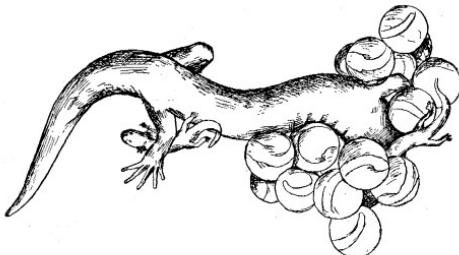


FIG. 6. Sketch of an adult female *Desmognathus fusca* with batch of hatching eggs. Drawn Sept. 25, 1907, by H. H. Wilder. Approximately life size.

The whole mass of soil was placed undisturbed in a small round glass terrarium, and the eggs, the remaining larva, and the adult were covered with some large wet leaves. A shallow crystallizing dish, filled with water and buried to the brim in the dirt at the bottom of the terrarium, was provided to take the place of the brook into which, if left undisturbed out of doors, the larvæ would presumably have found their way. Water was added to make the soil as thoroughly moist as in the natural habitat.

For five days the hatching continued at the rate of two or three a day. During this time the behavior of the mother and the young was frequently observed. In the course of the first 24 hours the position of the egg mass was entirely changed, probably owing to the gradual shifting due to the movements of the mother. After two days the mother no longer remained in contact with the eggs, but she stayed nearby in a burrow which she had made in the loose soil; she was usually found lying in

this with her head very near the hatching eggs. Little by little the newly hatched larvæ would stray from the nest, and would be seen, often in groups of two or three, disappearing into deeper and moister crevices in the loose soil. Often one or more would be seen in contact with the mother in her burrow. During the last two days of the hatching period, the mother was still deeper in her burrow, and although she may have returned to her nest unobserved, she never was found there. The larvæ, also, were vanishing, so that by the time the last of them hatched, only eight were visible in the nest.

Later, on October 1, search was made to learn what had become of the larvæ. Several were found in the loose mass of decaying leaves, in burrows which had been made by the mother, or in natural crevices in the loose soil. These were in groups of from two to four with the bodies in close contact. None were in the water in the glass dish; but upon lifting this, two were found in a little pool beneath it, a position which they had evidently reached by going down from the nest into the deeper and wetter layers of the loose debris until they reached the wet surface of the more solid layer at the bottom. In another little pool two others were found. These larvæ, like those in the loose soil above, were very active when disturbed. They placed themselves, as do the aquatic larvæ in their natural habitat, at the edge of the water with the head almost out and the back hardly covered. Eleven were found in all in different parts of the terrarium at this time. The others had gone down too far in the loose soil to be located without a more complete overturning of the terrarium than was deemed advisable. Five of the specimens were taken and preserved for later study (stages *B* and *C*). There is no means of knowing the exact date of hatching of each of these specimens, which may have been anywhere from one to five days old; it is very probable, however, that two of them, which were taken from the water (stage *C*) and which were somewhat larger than the others, were hatched earlier, especially as the others (stage *B*) were taken from near the nest.

During the next few days the terrarium was examined from time to time to locate the remaining larvæ and to discover any change in their activities. They never at any time showed the

slightest instinct to go directly to the little pool of water which was now arranged for their reception but would often leave the nest in exactly the opposite direction. In general they made their way, as has been noted above, deeper and deeper into the loose soil until they finally reached the little areas of very shallow water on the surface of the hard soil at the bottom. Under natural conditions the burrows which penetrate the soil in which the *Desmognathus* lives must furnish natural and easy channels for the descent of the larvæ to a level at which the burrows would contain little pools of water. From here it is an easy matter for them to make their way beneath the loose layer of vegetable debris to the open water of the neighboring brook. Possibly an instinct of the larvæ to remain in contact with the body of the mother serves as an additional guide to them, as they would thus naturally follow her movements through the burrows. That the larvæ, like the adults, are thigmotropic, would also lead them to confine their movements to the crevices in the soil; a negative heliotropism may also assist in determining the downward direction of their movements.

On October 5, five more specimens were killed (stage *D*); of these four were found to have been more or less mutilated, having suffered the loss of a tail, or one of the limbs or both tail and limbs. Since it frequently happens that a considerable proportion of larvæ collected from their natural habitat show mutilations similar to these, it is evident that the cause in question is one which is operative under natural conditions. It is possible that the injuries are inflicted by the stronger larvæ upon their weaker brothers, especially as the injured individuals are usually below the average size. It is more probable, however, that the mother, under the pressure of a paucity of food, found her offspring tempting morsels. The fact that two of the brood were shown by the final count to be missing suggests that this conjecture is the correct one, especially as the larvæ themselves are very hardy and will live for weeks in the laboratory under very adverse conditions. I have noted in several instances similar total disappearance of larvæ from terraria in which they had been placed in company with one or two adults, and in two cases have found whole larvæ in the stomachs of adults which were in the terrarium from which larvæ had disappeared.

On October 6 one specimen more was killed (stage *E*), and on October 14 all of the remaining ones which could be found, three in number (stage *F*).

Since by this time the larvae had not only reached the water in the bottom of the jar but had attained the size and proportions of the aquatic larvae collected from their natural habitat at this season of the year (cf. Table I., and Graphs I. and II.), it may be

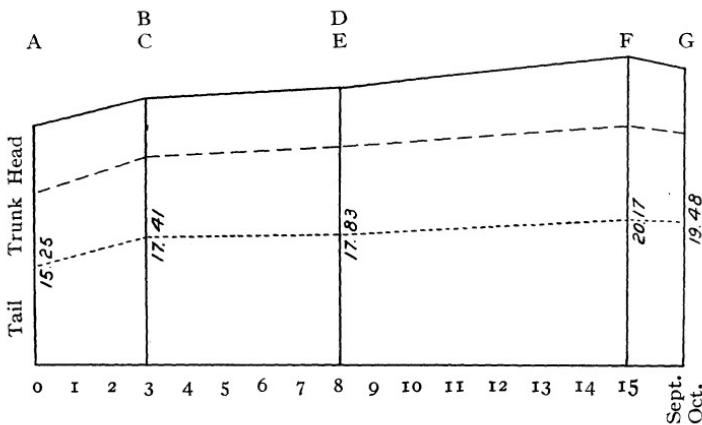
TABLE I.

(The measurements given in this table are approximately those of the head, trunk, and tail, respectively, though, for convenience in measuring, the axilla and the anterior angle which the posterior limbs make with the body were taken as the somewhat arbitrary limits of these regions, cf. Plate II., II, dotted lines.)

TERRESTRIAL LARVAL PERIOD.

Designation and Age.	Number of Examples.	Column I. Actual Sizes (Cf. Graph I.), Mm.			Column II. Proportionate Lengths of Body Regions (Cf. Graph II.)		
		Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.
Stage A. 0 days.	2	Head 4.00	4.00	4.00	.258	.267	.262
		Trunk 5.00	5.00	5.00	.323	.333	.328
		Tail 6.50	6.00	6.25	.419	.400	.410
		Total.....	15.50	15.00	15.25	1.000	1.000
Stages B and C. Average age 3 days.	5	Head 4.00	4.00	4.00	.219	.242	.234
		Trunk 5.50	5.00	5.33	.301	.303	.312
		Tail 8.75	7.50	8.085	.479	.454	.454
		Total.....	18.25	16.50	17.415	1.000	1.000
Stages D and E. Average age 8 days.	3	Head 4.25	4.00	4.17	.233	.229	.234
		Trunk 5.75	5.50	5.58	.315	.314	.313
		Tail 8.25	8.00	8.08	.452	.457	.453
		Total.....	18.25	17.50	17.83	1.000	1.000
Stage F. Average age 15 days.	3	Head 4.50	4.50	4.50	.220-	.225	.223
		Trunk 6.25	6.25	6.17	.305-	.3125	.306
		Tail 9.75	9.25	9.50	.476-	.4625	.471
		Total.....	20.50	20.00	20.17	1.000	1.000

considered that the duration of the terrestrial larval period is approximately 15 or 16 days, the average age of stage *F*. That the duration of the terrestrial period is very variable will, however, be inferred from the fact that in the case of this particular brood a difference in activity led some of the larvae to the water several



GRAPH I., showing the growth in length during the terrestrial larval period (cf. first column of statistics, Table I.). Lines A, BC, DE, and F correspond to the similarly designated stages, the average age of each stage in days being indicated by the number at the bottom of the line and the average length ($\times 2$) by the length of the line. Line G shows, for comparison with F, the average length of 42 aquatic larvæ collected during the months of September and October.

days in advance of others; and the differences in the distances of nests from the water would introduce still another variable factor.

While the limits of the terrestrial larval stage are somewhat indefinite, the structural changes which take place during this period are very important, and for the study of these changes the above described material may be tabulated as follows (see Plates I. and II.):

Stage A.—Two specimens killed September 25. Just hatched. Average length 15.25 mm.

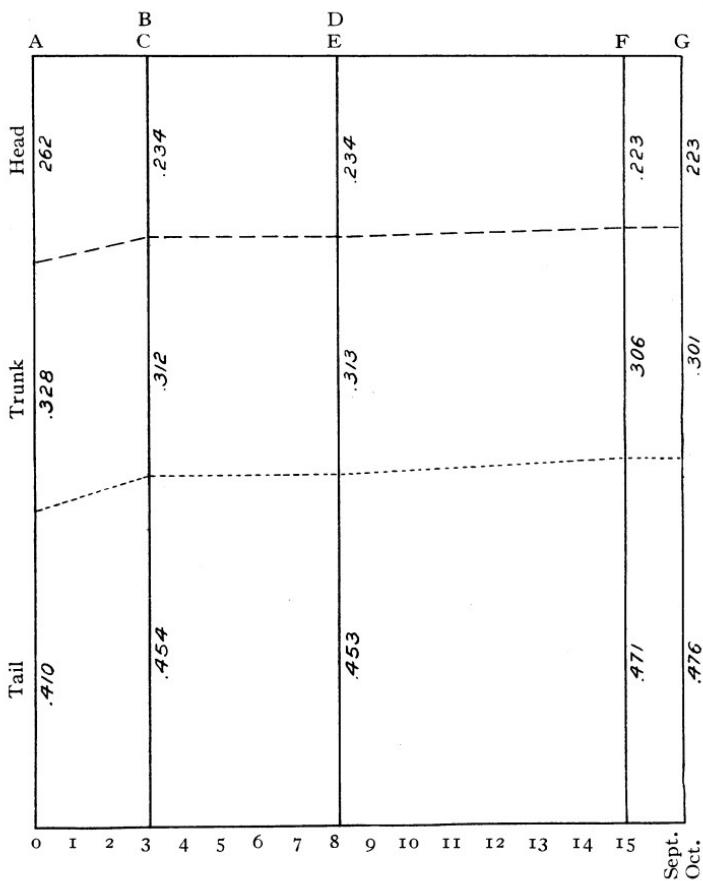
Stage B.—Three specimens killed October 1. Average age 3 days. As these were taken from near the nest the actual age of all of them is probably less. Average length 16.83 mm.

Stage C.—Two specimens killed October 1. Age as in the case of stage B, but as these two were taken from the pool of water at a greater distance from the nest, their actual age is probably above the average of 3 days. Average length 18 mm. Average length of B and C together is 17.415 mm.

Stage D. Five specimens killed October 5. Average age $7\frac{1}{2}$ days. Average length of the two uninjured ones 17.652 mm.

Stage E.—One specimen killed October 6. Average age $8\frac{1}{2}$ days. Length 18.25 mm. Average length of D and E together is 17.83 mm.

Stage F.—Three specimens killed October 14. Average age 15 days. Average length 20.17 mm.



GRAPH II., showing a comparison of the proportionate lengths of the body regions during the terrestrial larval period (cf. second column of statistics, Table I.). Lines *A*, *BC*, *DE*, and *F* refer to the various terrestrial stages as in Graph I. and line *G* shows, for comparison with *F*, the proportionate lengths of aquatic larvæ collected in September and October.

EXTERNAL FEATURES OF THE TERRESTRIAL LARVA.

The most conspicuous feature of the newly hatched larva (stage *A*, cf. Plate I., 1 and 2)¹ is the bulging form of the abdomen, due to the presence of a considerable mass of as yet unconsumed yolk material. The skin over this region is thin, unpigmented, and transparent, and the yellowish white appearance due to the yolk cells beneath it contrasts strongly with the deeply pigmented surface of the rest of the body. Along the back on each

¹ Plates will be found at the end of the second part of this paper in the next issue of the BIOLOGICAL BULLETIN.

side of the mid-line the general gray pigmentation is interrupted by a row of rather poorly defined, rounded areas of lighter color, in some cases showing a slight brownish tinge. These spots are somewhat irregularly arranged, usually not exactly opposite each other on the two sides. The number on each side in the trunk region varies from 7 to 10, and the series extends nearly throughout the caudal region where it finally leaves off by a gradual diminution in the size of the areas.

The tail is short and shows only a slight beginning of the dorsal and ventral folds which are later to form the tail fin. The gills are well developed and when the specimen is placed in water, they stand out conspicuously from the sides of the body. There are three of these gills upon each side. They are situated upon the lateral surface dorsal to the gill slits, in an oblique line, the most anterior one being more ventral in position and the most posterior one the more dorsal. This latter one is the longest and possesses from five to seven branches some of which bend conspicuously forward; the middle gill has usually five branches, and the ventral one three.

The legs, particularly the posterior ones, are stout and well developed, and are longer in proportion to the length of the trunk region than at any later period of development. Although the quick jerky motions with which the newly hatched larvæ, when disturbed, propel themselves through the loose soil seem to be performed with the whole body rather than with the legs, there are undoubtedly slower movements of the larvæ when left undisturbed, which involve the action of the legs. This greater proportionate size of the hind legs is, it will be remembered, one of the features distinguishing *Desmognathus* from *Spelerpes* larvæ, and is undoubtedly accounted for by the demands of this short terrestrial period preceding the aquatic larval life; since the eggs of *Spelerpes* develop, and the larvæ hatch, in the water, there is no need in this species for the early development of legs as organs of locomotion.

During the period of terrestrial life, important changes take place which result in a decided transformation in the appearance of the larvæ. The yolk protuberance rapidly diminishes, as will be seen by comparing Plate I., 1 to 9, so that by the time

stage *D* is reached (11 days, at the latest, from the time of hatching), practically all external evidence of the presence of yolk has disappeared, though dissection and sectioning both demonstrate the presence of an abundance of yolk granules in the walls of the intestine. With this reduction of the yolk protuberance, the medial edges of the *rectus abdominalis* muscles, widely separated at the time of hatching, gradually approach each other and finally meet in the *linea alba*.

The changes in the shape and appearance of the tail are noteworthy. The tail becomes longer, so that from being at

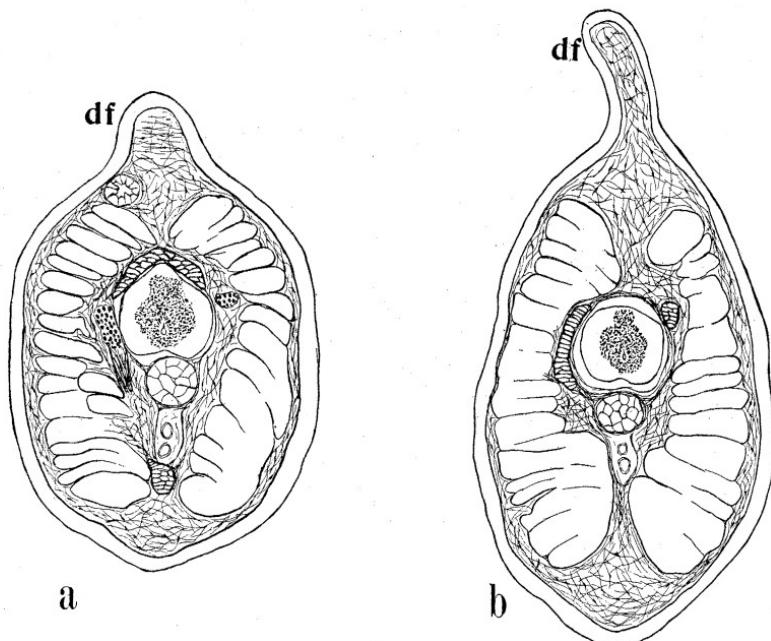


FIG. 7. Cross sections, at approximately the same level, of the tails of (a) a newly hatched larva, terrestrial stage *A*, and (b) an aquatic larva, showing the difference in proportions, and the development of the dorsal fin (*df*). For the level of the sections cf. Plate I., Figs. 1 and 10. Drawn with Abbé camera. X 60.

the time of hatching only 41 per cent. of the total body length, by the time the larvae have reached the water and become typical aquatic larvae (stage *F*, averaging only 15 or 16 days from the time of hatching) this same measurement is found to be over 47 per cent. of the total body length. With the increase in length of

tail comes the change in its shape owing to the development of the median fin both on the dorsal and ventral side. Dorsally it begins at the level of the posterior margin of the pelvic girdle, where a slight depression in the mid-dorsal line may be seen when the animal is viewed from the lateral aspect. This dorsal portion of the fin, which finally in its widest part comes to contribute from one third to two fifths of the entire dorso-ventral dimension of the tail, extends to the extreme tip of the tail, where its continuity with the ventral portion of the fin is interrupted by the slender tip of the vertebral column and its associated muscles. The ventral portion of the fin is neither so wide nor so extensive as the dorsal, and it narrows anteriorly to end at a point somewhat posterior to the cloaca. The increase in length of the tail is the principal factor in the change in length proportions of the body shown in the second column of Table I., and in Graph II. Thus while, during the terrestrial period, the head actually increases only about 12 per cent. in length, and the trunk 23 per cent., the tail increases 52 per cent. (cf. Table I., 1st column). These changes in the tail are obviously a preparation for the aquatic life which is so soon to follow.

Not only do the proportionate lengths of the regions of the body thus alter, but the actual growth is, during the first few days after hatching, very rapid, as will be seen from the 1st column of Table I., and also by comparison of Plate I., 1-9, all drawn to the same scale. Thus at the end of 16 days the total increase in length ($20.17 - 15.25 = 4.92$ mm.) is 32 per cent. of the length of the body at the time of hatching.

THE ALIMENTARY CANAL OF THE TERRESTRIAL LARVA.

Internally no structure exhibits more striking and fundamental changes during the terrestrial period than the alimentary canal. In the walls of the alimentary canal at the time of hatching there is still a considerable supply of yolk material, although several days previous to hatching (*i. e.*, in the embryo of 30 days' development, 13 mm. long), the yolk material contained in the cells of the other tissues has been already quite consumed. In the newly hatched larva, indeed, the digestive tract has so far progressed

in its development that oesophagus and stomach have already completed their histogenesis, and the duodenum and rectum practically so; leaving the yolk material in the remaining intestinal region.

The lining of the oesophagus is thrown into longitudinal folds, and is composed of columnar cells of the ciliated type with a large number of active unicellular glands interspersed among them (Fig. 8, *b*). The stomach is sharply differentiated from the oesophagus on the one hand and from the duodenum on the

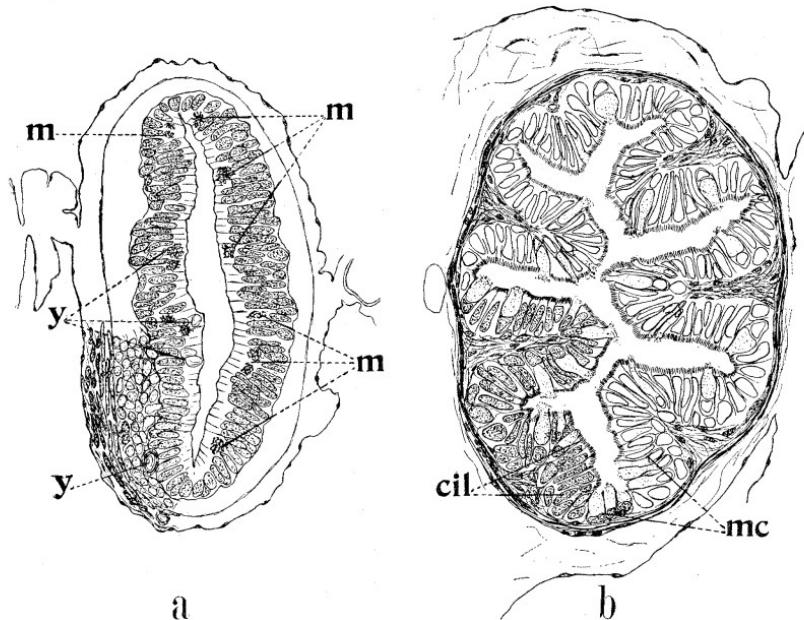


FIG. 8. Cross sections of the oesophagus of (*a*) a 13 mm. embryo, and (*b*) a newly hatched larva, showing the change in the character of the epithelial lining which takes place during the few days previous to hatching. (*a*) *m*, mitosis; *y*, last traces of yolk granules in the epithelial cells; (*b*) *cil*, ciliated epithelial cells; *mc*, mucous cells. Note the absence of folds in (*a*), and of mitosis in (*b*). Drawn with Abbé camera. $\times 103$.

other. It is divided into cardiac and pyloric regions, the former expanded, piriform, and glandular, the latter tubular and far more muscular. In both these regions of the stomach the unicellular type of gland is lacking, but there are numerous multicellular glands of the convoluted tubular type, opening

between the many irregular folds of the lining mucous membrane. The cells of the mucous membrane are sub-columnar in form, with a smooth exposed surface. The final stages of this histogenesis must take place with great rapidity, during the last few days before hatching. In the 13 mm. embryo neither folds, ciliated cells, nor glands of any kind have appeared in the alimentary canal; the stomach is differentiated from the oesoph-

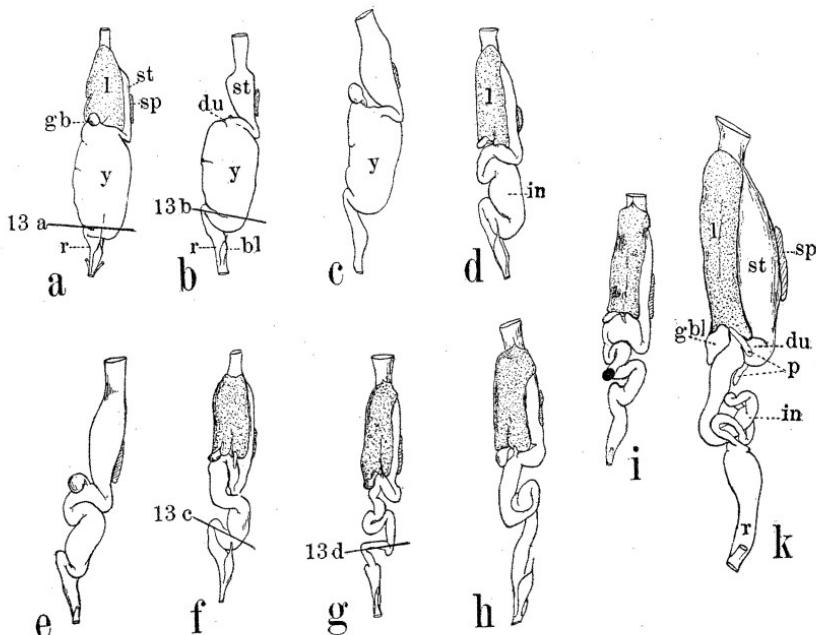


FIG. 9. A series of dissections of the alimentary canals of various stages of *Desmognathus fusca*, showing the gradual consumption of the yolk and the differentiation of the intestine. (a) terrestrial larval stage B, 3 days old; (b) same stage as (a) with the liver removed; (c) terrestrial larval stage D, 7½ days old; (d) terrestrial larval stage F, 15½ days old; (e), (f), (g), (h), aquatic larval stages collected in September and October; (i) aquatic larval stage collected in May; (k) adult stage, length 45 mm.

Lines 13a, 13b, 13c, and 13d show the levels and directions of the respective sections of Fig. 13; bl, bladder; du, duodenum; gbl, gall bladder; in, intestine; l, liver; p, pancreas; st, stomach; sp, spleen; r, rectum; y, yolk mass. Drawn with Abbé camera. X 4.

agus, only in being less flattened dorso-ventrally, and containing in its cells a larger amount of yolk; there is an enormous amount of mitosis in progress especially in the oesophageal

region (Fig. 8, *a*), undoubtedly the process of cell proliferation which results in the formation of the longitudinal folds.

With equally rapid strides does the histogenesis of the intestinal region occur during the brief terrestrial period of larval life, until from the large yolk mass which fills and distends the body cavity of the newly hatched larva and furnishes nutriment for the rapidly growing animal, there is developed the entire length of intestine with its convoluted windings, ready for the digestion and absorption of ingested food material.

The changes in the external form of the intestinal region involved in this process may be demonstrated by comparison of the dissections shown in Fig. 9, from which it will be seen that there is gradually moulded out of the intestinal yolk mass a loop forming an *S*-shaped figure which consists of an anterior portion lying upon the right side, into which the duodenum opens, and a posterior portion lying upon the left side, from which the rectum leads. Both of these regions, particularly the left and more posterior loop, are for a considerable time enlarged through the presence of the remaining yolk material as indicated by the condition shown in stage *F*, (Fig. 9, *d*) the oldest of the terrestrial larvæ. Gradually, however, concomitant with the consumption of the last remnants of yolk material and the consequent diminution in the diameter of the intestine, there occurs a still further increase in its length, so that each of the two main loops acquires two or three subsidiary ones variable, of course, in relative position with constantly varying peristaltic conditions (cf. Fig. 9, *e*, *f*, *g*, and *h*).

The last part of this process of differentiation of the intestine is apparently not usually accomplished until after the larva has reached the water, since frequently in the early fall aquatic larvæ are found with the intestine in the condition shown in *d*, *e* and *f*, Fig. 9, exactly similar to the condition found in my oldest terrestrial stage *F*. Food may be ingested many days previous to the final disappearance of the yolk; in fact, the stomach of one specimen of stage *C* (terrestrial larva, average age 3 days and greatest possible age 5 days) contained a small copepod, while there was another in the intestine; and the specimen of stage *D*, from which the dissection shown in Fig. 9, *C*, was made, had 30

copepods in the stomach, and in the stomach of another specimen of the same stage 6 copepods were found.¹ In all the cases which I have examined, of aquatic larvæ in which yolk was still present, feeding has been extensive and fragments of partly digested aquatic animals, in the main copepods and insect larvæ, have been found not only in the stomach but in various regions of the intestinal tract. Whether the early assumption of its digestive function on the part of the alimentary canal has the effect either to retard the final consumption of yolk material through the supply of nutriment from external sources, or to hasten its consumption through the stimulation of the histogenesis of the walls of the canal through use, I am at present unable to say. At any rate the excess of the supply of yolk material over the amount which the larva usually consumes before reaching the water with its ample supply of appropriate food, is undoubtedly an adaptation to a possible prolongation of the terrestrial period such as might easily occur if the eggs happened to be laid at a greater distance than usual from the water, or if an unusually dry season during the period of development of the egg should diminish the water supply in the brook and in the burrows leading to it and thus lengthen the period which might elapse before the larvæ should reach the water.

Internally the differentiation of the intestine from the mass of yolk cells is a somewhat complicated process, the detailed account of which must be reserved for a more technical treatment of the whole subject of the histogenesis of the alimentary canal. Certain salient features of the process may, however, be discussed here.

At the time of hatching the duodenum is in what may be termed the transition stage in its development (*d*, Fig. 12). Anterior to the point where the bile duct leads into the duodenum from the fully formed liver, yolk granules have disappeared, as they have also from the cells of the liver and pancreas and their ducts. Posterior to the opening of the bile duct into the duodenum, there is for some distance a well-defined lumen, but the epithelial cells lining it contain a considerable number of yolk

¹ All of these individuals, though ranking as terrestrial larvæ, happen to be among the more precocious ones which had reached the water, where they had access to food.

granules, although among these cells there have already appeared well-developed mucous cells which are filled with their secretion and are already discharging it into the lumen. The duodenum leads rather abruptly into the right anterior region of the cylindrical yolk mass, beneath the posterior margin of the liver.

In this mass of yolk cells two types of cell may be distinguished, a smaller and a larger (*ys* and *yl*, Fig. 11). The smaller ones lie peripherally, have a spherical or slightly columnar form with a well-defined nucleus usually in the peripheral end of the cell.

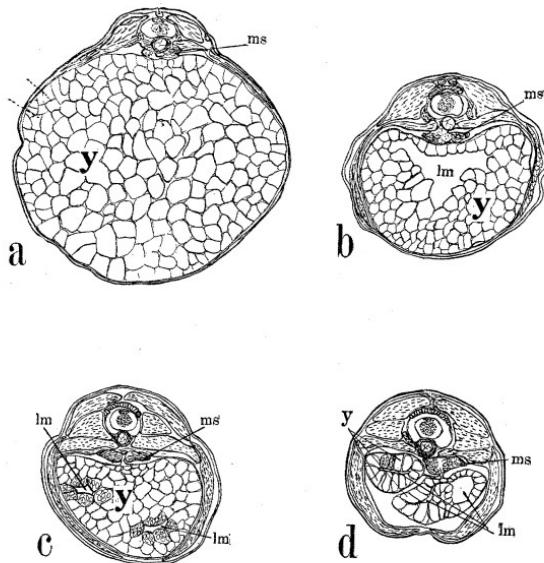


FIG. 10. Cross sections at the same level through the middle of the body of (a) a 13 mm. embryo; (b) newly hatched larva, terrestrial stage A (cf. Fig. 12, a, and Plate I., Figs. 1 and 2); (c) larva 3 days old, terrestrial stage C (cf. Fig. 12, c, and Plate I., Figs. 5 and 6); (d) larva 15 1/2 days old, terrestrial stage F (cf. Fig. 12, d, and Plate I., Fig. 9). Note the gradual reduction of the mass of yolk cells (*y*) and the formation of the intestinal lumen (*lm*); *ms*, mesonephros. Enclosure between the dotted lines in (a) indicates the location of Fig. 11. Drawn with Abbé camera. $\times 12\frac{1}{2}$.

The cell walls are definite, the contained yolk granules of varying size, but among them relatively few large ones. The remaining portion of the yolk mass consists of enormous cells which are often multinucleate, with very large, irregular nuclei. The cell walls are not always clearly defined and in many regions seem

to rupture easily. One must, however, remember the great liability to imperfect preservation of the middle region of the yolk, which might account for apparent imperfection of cell walls. Although during the earlier part of development within the egg, these yolk cells of the large type contain both large and small yolk granules with, however, a preponderance of the large ones, by the time the larva hatches practically all of the smaller granules seem to have been consumed, and as a result the large granules alone appear, and these not very closely crowded together. It is from the cells of the smaller type, the

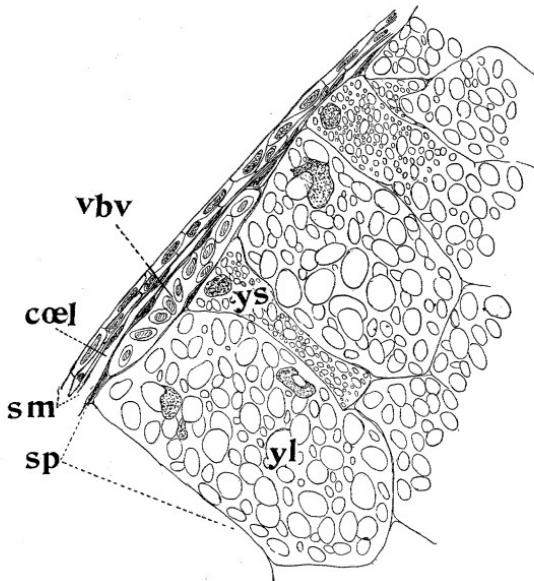


FIG. 11. Detail of region indicated between the dotted lines in Fig. 10 (a). *Cœl*, coelomic cavity; *sm*, somatopleure; *sp*, splanchnopleure; *vbw*, vitelline blood vessel; *yl*, yolk cells of the larger (nutritive) type; *ys*, yolk cells of the smaller type. Abbé camera. $\times 273$.

peripheral ones, that the future epithelial lining of the intestine will arise, while the large cells which form the bulk of the yolk mass may be regarded as purely nutritive in function, since from these apparently no tissue is derived. If this distinction goes back, as seems probable, to the early differentiation of the cells of the blastula into the peripheral ones containing small yolk granules, and the central mass of cells containing large yolk

granules (Hilton, '09), we see again the approach to an intermediate position of the *Desmognathus* egg between the holoblastic and meroblastic types, this mass of large cells of a purely nutritive character being comparable to the undivided yolk mass of the meroblastic egg. The tardy segmentation of this mass of

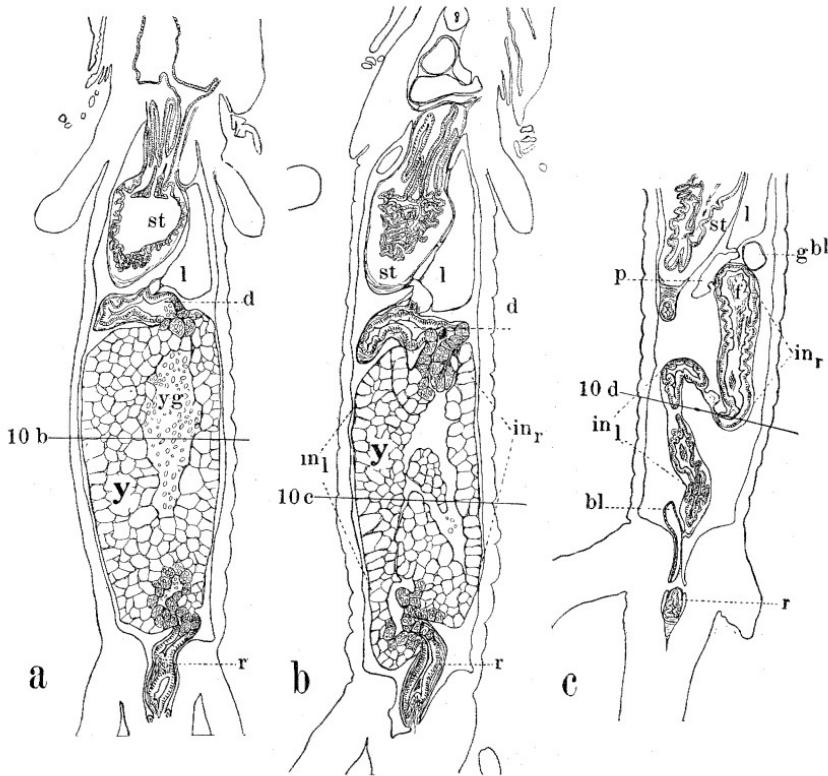


FIG. 12. Horizontal sections (viewed dorsally) of (a) terrestrial larva at time of hatching (stage A, cf. Plate I., Figs. 1 and 2), (b) terrestrial larva 3 days old (stage C, cf. Plate I., Figs. 5 and 6), (c) terrestrial larva $15\frac{1}{2}$ days old (stage F, cf. Plate I., Fig. 9). Lines 10b, 10c, and 10d indicate respectively the levels of the cross sections shown in Fig. 10. Bl, bladder; d, duodenum; gbl, gall bladder; in_l, left loop of intestine; in_r, right loop of intestine; l, liver; p, pancreas; st, stomach; r, rectum; y, yolk mass; yg, yolk granules in the lumen of the yolk mass. Drawn with Abbé camera. $\times 12\frac{1}{2}$.

yolk material, which Hilton pointed out, and the later breaking down of the cell walls of these large nutritive cells, which seems to appear in some cases, corroborate this view.

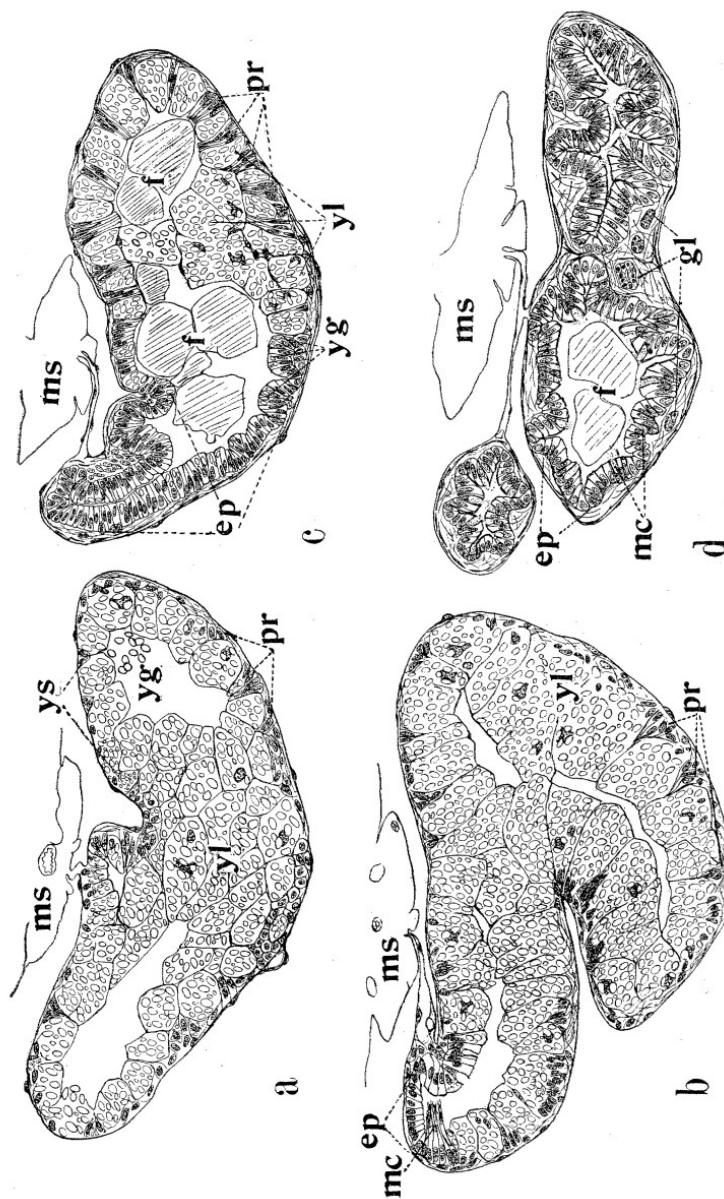


FIG. 13. Sections showing four stages on the differentiation of the posterior part of the intestine from the mass of yolk cells, from cross sections through approximately the same region of the body; (a) terrestrial larva at the time of hatching, (b) terrestrial larva 3 days old, (c) and (d) two aquatic larvae collected in September. The levels and the directions of the sections with respect to the yolk mass and intestine are indicated approximately by lines 13a, 13b, 13c, 13d, respectively, in Fig. 9 (a), (b), (f) and (g). Ep, regions of functional epithelium; f, food in the intestinal tract; gl, multicellular glands of the intestinal mucous membrane; mc, mucous cells; ms, mesonephros; pr, regions of proliferation of epithelial cells to form future intestinal folds; yg, yolk granules; yl and ys, yolk cells of the large (nutritive) and small (tissue-forming) types respectively. Drawn with Abb écamera. $\times 60$.

Although there is at the time of hatching a considerable cavity within the yolk mass (Figs. 10, *b*, and 12, *a*), its walls are irregular and poorly defined. The anterior region of the cavity is located more upon the right side and communicates with the lumen of the duodenum, while more posteriorly the cavity swings somewhat to the left side, where it finally communicates with a partly differentiated posterior region of the intestine which extends transversely from left to right to lead into the rectum. Thus the S-form so soon to appear in the external moulding of the intestine (Fig. 12, *c*) is already indicated in the lumen of the yolk mass.

The further differentiation of the intestine from the mass of yolk cells (Fig. 13) involves a double process consisting of (1) rapid reduction and disappearance of the yolk material contained in the nutritive cells (*yl*), which may from time to time be sloughed off into the lumen, or may be ruptured, thus discharging their contents into the lumen; and (2) the proliferation of the peripheral cells (*pr*), the planes of division being radial with relation to the lumen of the intestine, and resulting in the formation of the anlagen of the future folds of the mucous membrane, in the form of groups of tall columnar cells which, elongating, push their way to the lumen between the remaining nutritive cells (Fig. 13, *c*). These nutritive cells thus come to lie in the furrows between the folds, from which place they gradually disappear. Mucous cells develop early from certain of the peripheral cells, and are of a tall columnar type, corresponding to the form of the other epithelial cells among which they lie. Multicellular glands (*gl*) develop later from a proliferation of cells at the bases of the folds.

Although this differentiation of the intestine progresses almost simultaneously throughout the main part of the yolk mass, it proceeds a little more rapidly at both anterior and posterior ends, than in the middle of the yolk region. Thus at the time of hatching the anterior part of the duodenum and practically all of the rectum are fully differentiated, and the regions of intestine immediately adjoining these are in that stage of development in which the anlagen of the intestinal folds are already formed, a condition which is not reached by the larger portion of the intestine until several days later.

THE SKIN OF THE TERRESTRIAL LARVA.

The epidermis of the newly hatched larva (Plate IV., 19, 20, 21, and 22) is in the typical larval amphibian condition in that it consists of two layers of cells; these are for the most part subspherical in shape with polygonal outlines when viewed in a section parallel with the external surface. They possess large nuclei which in both layers show a small percentage of mitosis. The cells of the outer layer have a deeply staining cuticular border on the exposed surface, forming all together a continuous cuticular layer (*cu*) covering the external surface. In general over the dorsal and dorso-lateral surfaces the outer layer of the epidermis is the thinner one and its cells are slightly flattened. Over the lateral and ventral surfaces the outer layer is the thicker, the cells here approaching the columnar form, while their nuclei are piriform with the pointed end directed toward the outer surface and often nearly reaching it; in these regions the inner layer is considerably thinner and the nuclei smaller. Over the region which is still distended with yolk, both layers are thin, the cells of the inner one approaching the squamous type. In a few limited regions as, for example, the lateral wall of the body beneath the gills, the whole epidermis is very thin, and the cells of both layers decidedly of the squamous type; as seen in cross section their nuclei alternate, and the whole appearance may thus even simulate a single cell layer.

Beneath the deeper layer of epidermal cells in practically all regions of the body except the snout, there is a dense corium which fits closely to the deeper layer of cells, thus following their contour. This corium is a little thicker than the cuticular layer of the outer cells and is from one eighth to one sixth as thick as the whole epidermis.

In those regions of the body which are pigmented, the dorsal and lateral surfaces, the pigmentation is threefold. The external layer of epidermal cells possesses an intracellular pigmentation in the form of a layer of pigment granules (*pgg*) immediately beneath the cuticular layer and forming a sort of cap over the nucleus. In addition to this intracellular pigmentation there may be seen, between the cells, delicate intercellular ramifying branches (*pgbr*) of pigment cells (*pgc*) of the connective tissue

type, the body of the cell often lying between the two layers of epidermal cells. These branches seem particularly to embrace the cells of the external layer, though occurring between the cells of the deeper layer also. Finally, beneath the corium and closely applied to it, is an interrupted layer of enormous patches of pigment matter which, however, does not seem to penetrate the corium and encroach upon the epidermis. In the unpigmented ventral region all of these forms of pigmentation are lacking.

In comparison with the skin of the 13 mm. embryo the skin of the newly hatched larva of 15.5 mm. impresses one as a fully formed structure (Plate IV., 17 and 18). In many regions of the 13 mm. embryo the epidermis is very thin, and although two layers of cells are present, the nuclei, particularly of the outer layer, are often so widely separated, owing to the flattened form of the cells, as to seem in a vertical section through the skin to be entirely lacking for considerable distances (cf. Fig. 11). There is only the merest trace of cuticular structure in the external border of the outer cells, and practically no corium beneath the lower cells. Branched pigment cells occur both beneath and between the epidermal layers but there is no evidence whatever of intracellular pigmentation of the epidermis. The embryonal character of the epidermis is evidenced by the slightly rounded external contour of the outer layer of cells, and by the large size of the nuclei, which, moreover, present an enormous amount of mitosis (*mt*) in both layers. As might be expected from the fact that the larval two-layered condition has already been reached, the mitotic figures are oriented with their axes parallel with the external surface of the body, and the process is, of course, giving rise to an increase in the number of cells in each layer. This condition of general mitosis contrasts sharply with the limited amount of mitosis in progress in the epidermis of the newly hatched larva, where the figures are, moreover, in late anaphase or telophase.

There occur after hatching but few changes in the skin of the larva to complete this rapid transformation from the embryonal condition to that which is to serve the animal during its eight or more months of larval life (Plate IV., 23, 24, and 25). These

changes, which take place during the brief terrestrial period, involve no mitosis, not even in the rapidly growing tail and tail fin. They consist, rather, in a readjustment and rearrangement of the cells to suit the changes in the shape and proportions of the body, the full quota of cells having been already formed in the mitotic stage preceding hatching. Thus over the growing tail fin the epidermal cells become stretched apart, the cells of the deeper layer alternating with those of the external layer and in some cases actually reaching the external surface. In the ventral region of the body where the area of the surface is rapidly diminishing with the reduction of the yolk, the cells lose their flattened form and become subspherical or even columnar.

Over all parts of the body with limited exceptions, there is a slight increase in the thickness of the skin from various causes. The cuticular layer of the external cells increases slightly in thickness, probably, however, at the expense of the rest of the cell; the dense corium underlying the epidermis becomes a little thicker; the chief increase comes, however, from that change in the inner layer of epidermal cells which is the most conspicuous change in the skin during the terrestrial larval life, the distension of the cells as if from a condition of turgor. This distended condition soon becomes more marked in certain cells than in others, and these eventually form the greatly enlarged, vacuolated cells of the type described by Leydig ('53) and subsequently designated the "Leydig cells" of the epidermis (*lc*). These show a somewhat reduced nucleus and a large cytoplasmic region in which a loose reticulum sometimes appears, but which with most stains gives the effect of a clear area between the nucleus and the thin cell membrane. This enlargement of certain cells naturally increases the thickness of the deeper epidermal layer. The Leydig cells press inward upon the corium and outward upon the cells of the external layer; they seem even to push around and between these latter cells but never to actually reach the external surface. The remaining cells of the deeper layer, which lie between the Leydig cells, become crowded together into a columnar form and reach in most cases from the corium to the outer layer of cells. The differentiation of the Leydig cells has already begun at the time of hatching and is most conspicuous

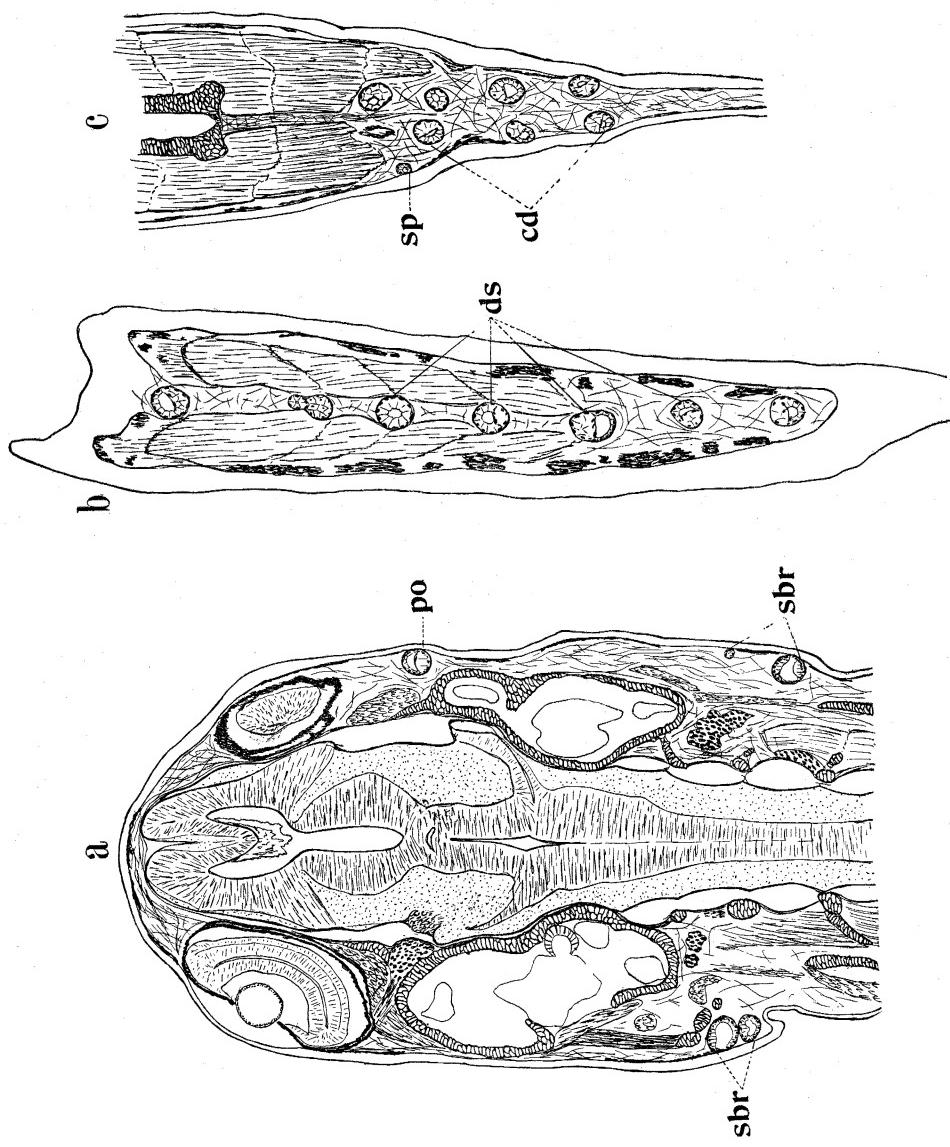


FIG. 14. Horizontal sections of *Desmognathus fusca* larvae showing the distribution of larval acinous glands; (a) through the post-orbital (*po*) and the suprabranchial (*sbr*) groups of glands; (b) through seven of the mid-dorsal (*ds*) group; (c) through a portion of the double row of the tail (*cd*) at the level of the base of the dorsal fin; also showing a single sporadic gland (*sp*). Drawn with Abbe camera. X 32.5.

along the dorso-lateral regions; it proceeds so rapidly during the few succeeding days that in stage *D* the process is almost completed.

Two sets of highly specialized integumental organs have already made their appearance at the time of hatching. One of these consists of the neuromasts, or sense organs (*sn*) of the lateral line type, which are abundantly distributed over the head, especially the snout, and in two lines which extend along each side of the trunk, while a single line upon each side extends along the tail. These organs, each of which consists of a group of specialized epithelial cells, begin their development long before hatching and at the time of hatching appear to be fully formed so far as the number of cells entering into them is concerned. The surrounding accessory cells probably undergo some further modification.

The other organs to be mentioned are multicellular glands of the alveolar type. They are limited in number and occur in definite locations; a small group of two or three posterior to each eye (the post-orbital group, *po*, Fig. 14, *a*), a group of from one to three over the otic region (the supra-otic group), a larger group of from six to eight on each side of the head posterior to the otic region and dorsal to the gill bushes (the supra-branchial group, *sbr*, Fig. 14, *a*), and a single row approximately segmentally arranged along the middorsal line of the trunk (*ds*, Fig. 14, *b*), becoming, in the transition to the caudal region, a double row in slightly alternating, segmental pairs (*cd*, Fig. 14, *c*) lying along the line of junction of the dorsal finfold and the tail. With the exception of those at the posterior end of the caudal series and a few of the supra-branchial group, these glands are very large, measuring in diameter from two to eight times the thickness of the epidermis; and although they have developed from the epidermis they lie quite beneath both the epidermis and corium within the loose layer of subcutaneous tissue, and open to the exterior by a slender duct which leads through the skin (Fig. 15, *a*). Some of them have already made their appearance in the 18-day embryo as a mass of rapidly dividing epithelial cells, and in the 13 mm. embryo of 30 days' development are

already discharging a granular secretion upon the surface of the skin. Thus the larva hatches fully equipped with a set of actively secreting glands which are to continue their activity throughout the larval life.

(Concluded in the next issue.)